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EFFECT OF VARIOUS BIOCATALYZERS ON THE WATER-PERMEABILITY OF PROTOPLASM

/Following is a translation of an article by Hermann von Guttenberg and Anna Beythien of the University of Rostock Botanical Institute, Rostock, Germany, in the German-language publication Planta, Vol. 40, 1951, pages 36-39./

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I. Introduction

The writers stated in an earlier work (Krŏpelin 1945, Von Guttenberg and Krŏpelin 1947) that the pulvinus of Phaseolus Multiflorus carried out movements which correspond to the normal nastic movements of such plants whenever heteroauxin or growth factors of natural origin (from Avena, for example) are applied externally to the plant or taken in, internally. Possible explanations offered for this behavior were: changes in the elastic or plastic properties of the membrane; changes in the pressure deficit or suction tension of its contents or an effect on the water-permeability of the pulvinus cells.

It was assumed for a long time by a number of authors (such as Von Overbeck 1926, Söding 1931, Heyn from 1931 onwards and Ruge 1937) that growth factors affected the membrane directly. The reason which led one to hold firm so long to such an improbable interpretation was the fact that though changes were observed taking place in the protoplasm and the cell sap, they always occurred only after a certain period of time had elapsed, which was a great deal longer than the reaction time of the growth process. Thus Friedrich (1936) did find an increase in sugars present on the

under side of Helianthus shoots when stimulated geotropically and in this case enriched with growth factors, but this did not make its appearance until after a number of hours had passed. According to Ruge, too, certain physiological changes in the cells only occur a considerable period of time subsequent to the growth reaction. More recently Renders (1942) demonstrated that there is an increase in respiration which sets in under the influence of growth factors and that this leads after a passage of a number of hours and even after some days to an increase in osmotic substance.

No tests had as yet been made by anyone to determine what effect growth factors might have on protoplasm permeability. Because of this we investigated in the above mentioned paper, the possibility that the water-permeability of protoplasm might change under the influence of growth factors. We selected the acceleration of deplasmolysis time (Huber and Höflor 1930) as a measure of increased water permeability. Since the pulvinus cells of the leaf joint are poorly suited to such experiments, we selected two well-proven subjects for our experiments, namely, the epidermal cells of the lower leaf side of Rhoeo discolor and of onion skins. An increase in water permeability brought about by growth factors could then be proven valid if the deplasmolysis time were decreased in such subjects as compared with controls.

We did, in fact, discover the existence of such an acceleration specifically upon utilizing heteroauxin or concentrations of diffusate lying within known physiological limits.

These investigations had to be broken off permaturely because of the events of 1945 (immediate aftermath of World War II). The results attained appeared important enough, though, to prepare the way for taking the experiments up again on a broad basis. The primary effect of the growth factor, so long sought after, was discovered and it was recognized that it concerned neither the protoplasm nor the cell wall. Particularly important was the fact that the protoplasm was affected in a very short time, thereby explaining unusual speed with which heteroauxin carried out its action as had also been reported shortly beforehand by Sutter (1944) in her work. After our first report (Krŏpelin 1945) another paper by Koningsberger (1947) appeared establishing the existence of a temporary total inhibition of deplasmolysis on the part of heteroauxin 10^{-7} and 10^{-6}

in isolated protoplasts of onion skins, lasting seven to nine minutes; this phenomenon will be dealt with further later on in this paper. Veldstra (1944) also seized upon this concept and even identified the growth factors as being regulators of protoplasm permeability. Finally, Pohl (1949) investigated the effect which growth factors have on the elongation of coleoptiles. He found that the sudden elongation of the cells requires an increase of from four to six times in the cell's suction tension. This increase cannot be attributed solely to the release of pressure by the cell walls, since the latter amounts to only one-fourth to one-tenth of the cell suction tension. On the other hand, it has been brought to light, particularly in investigations by Ruge, that the suction tension of the cell contents does not increase during cell elongation but rather decreases considerably. A further factor must therefore be responsible for the rapid water uptake, and the question arose as to whether an increase in permeability to water might not be the cause. Later on several additional new papers will be dealt with.

The part which follows describes the effects which different growth stimulants and inhibitors as well as other agents have on the water permeability of protoplasm. Supplementing this work we have also carried out tests with K^{+} and Ca^{++} ions, as had been done earlier particularly by Fitting (1915), Gellhorn (1930) and deHaan (1935). Lastly, growth stimulants and inhibitors were combined and added in the hope that the results of such experiments might enable us to reach further conclusions as to the manner in which these substances carry out their action.

II. Materials and Methods Used

Preliminary tests indicated that the violet epidermal cells of Rhoeo discolor would prove most satisfactory as test subjects. Here, too, certain additional precautionary measures had to be taken in order to be able to work with material which could readily be compared. The epidermal cells of the middle third of the leaf showed themselves of equal value from the standpoint of osmosis. The leaf tip and base showed large differences in osmotic properties. The epidermal strips were taken near the median portion of the leaf and square surface cuts one to two mm. long on one side were made with razor blade to remove pieces as thin as possible. Each such piece comprised about 80 to 130 intact cells. Test runs were carried out only on cells from the same area so the condition of the protoplasts could be

generally considered as identical. Each run of tests was observed through a number of microscopes side by side in a row. This ensured that the same temperatures and light intensities would be present. These factors are important because in accordance with our observations, we found that differences or fluctuations in these factors cause the deplasmolysis times to change. Such times become longer with lower temperatures and decreased illumination, particularly in the evening, while an increase in temperatures brings about considerable acceleration. These finding concord with figures given by Derry (1930) who found that plasmolysis time was shorter in Spirogyra the higher the temperature rose. Since we were unable to keep the temperature and light constant at all times we averaged the figures of the sixth to tenth test runs of a single day and registered the results in the tables. Along with the fluctuations in readings caused by light and temperature, we discovered also the existence of seasonal and climatic effects (for example, effects induced by stormy weather). According to Fitting (1919) and Bärlund (1929) plasmolysis, itself, does not bring about any change in the protoplasm permeability of Rhoeo.

Mannitol showed itself to be preferable as a medium for plasmolysis, since the Rhoeo protoplast is not permeable to this substance. It was found in many endurance tests that even after 40 hours duration in the mannitol solution, plasmolysis failed to recede. A 0.5 molar solution of mannitol in distilled water served as a stock solution. Plasmolysis was for the most part carried out with a 0.3 molar solution and deplasmolysis with a 0.2 molar solution. The individual concentrations conformed to the osmotic properties of the particular Rhoeo epidermis being tested. This was determined each time by determining the limit of plasmolysis, using graduated mannitol solutions differing from one another in 0.0025 mol steps.

In general the work was carried on with a number of test runs simultaneously. Pure mannitol solutions were used for the plasmolysis and deplasmolysis test runs on controls. Both kinds of solutions of the agent to be tested were used on the experimental runs, using graduated contrations. First the cut out pieces remained for 30 to 60 minutes in covered glass containers in the plasmolytic medium. Then they were transferred into the deplasmolytic medium using a platinum loop and were bathed in it there for from two to five minutes to remove any remaining plasmolytic medium. Subsequently they were placed on a hollow

ground slide with deplasmolytic medium under the microscope and were left there for one minute in exposed droplets before starting the first counting. We then put it back in the deplasmolyzing solution. This process was then repeated again at intervals of several minutes until just before total deplasmolysis set in and the counting was done under a cover glass supported by glass fragments. Thus the test pieces were always fully washed and rinsed, were not cut off from the air and the solution was protected against evaporation.

At first we used the method of evaluation according to Kaczmarek (1929), which was based on earlier results obtained by Fitting (1915). In this method of calculation all the violet cells in a single section are counted and the number of plasmolyzed cells at the present stage of the test is given as a percentage of the total number of cells. The numbers therefore increase during the course of the plasmolysis test up to 100, while during deplasmolysis they decrease until finally zero is reached, i.e., until all the membrane's cells are again in contact with the membrane. Later we used the more simple and vivid method of calculation described by deHaan (1935). The procedure used by deHaan consists in defining the quotient resulting from the deplasmolysis time of the controls = Tk and the deplasmolysis time of the test subjects = Tv. If Tk = Tv, then the quotient is 1 and the solution being tested has no particular effect. The accelerated effect of a substance is indicated by a quotient larger than 1 and the retarding effect is shown by a quotient smaller than 1. The absolute times of these tests corresponded to the earlier tests, i.e., the deplasmolysis times of the controls fluctuated according to the temperature between about 20 and 40 minutes. A lasting inhibition of deplasmolysis is indicated in the tables by a dash. These are those special cases in which the protoplasts never did return as far as the cell wall at all. A+ in the tables means damage to or destruction of the protoplasts.

III. Test Results

a) Growth Factors

1. Heteroauxin. Beta-indolylacetic acid manufactured by the Merck Co., Darmstadt, Germany, was used for the tests with heteroauxin (abbreviated HA in the text which follows). The substance was dissolved at 40°C., was kept in brown glass bottles in a cool place. The solutions were

used no longer than three days because they soon began to decompose (pale pink coloration). The concentrations of HA given in the table are in grams per milliliter. Tests were carried out between 10^{-3} (one part in one thousand) and 10^{-7} (one part in ten million). The 10^{-3} concentration brings about complete destruction of the protoplasts in a short time, which will be reported on in greater detail later on. It will suffice to mention here the fact that the changes which were noted to take place in these tests and in those which followed were not brought about by any change in pH, but represent specific effects in themselves. The pH value was first tested by Dr. Höll with the aid of litmus paper having an accuracy of 0.2 pH. Later on we used an ionization gauge with quinhydrone elctrodes and an accuracy of pH 0.1. The detailed results of this investigation will be reported in a subsequent paper. Tables 1 and 2 show the results of plasmolysis and deplasmolysis tests using the Kaczmarek system of evaluation. On 16 June 1949 the HA was added to the deplasmolytic medium only, hence the plasmolytic medium did not contain HA. It will be noted in table 1 that after 10 minutes in the control solution upwards of 45% of the cells were plasmolyzed and after 40 minutes all the protoplasts were again in contact with their cell walls. Upon addition of HA one noted after 10 minutes between 10^{-4} and 10^{-7} an increasing and finally a very powerful inhibition of the entry of water into the cells. If we disregard the effects of concentrations of 10^{-4} and greater, one finds that such an inhibition is only transitory. With 10^{-5} it still lasts a while, but at 10^{-6} and 10^{-7}, i.e., in those concentrations, most significant from a physiological standpoint, there is a sudden reversal. The water now enters the cells so rapidly that at 10^{-7} all protoplasts reach their cell walls after only 20 minutes (compared with 40 for the controls). This shows that the water permeability of the protoplasts greatly increases within the range of concentrations of HA.

Tabelle 1. (a)

Deplasmolyse. (b)

16. 6. 49.

Zeit min (c)	Kontrolle (d)	Versuch mit Heteroauxin (e)			
		10^{-4}	10^{-5}	10^{-6}	10^{-7}
10	45	62	75	80	88
15	28	50	50	13	0
20	22	40	31	4	0
25	11	40	10	2	
30	6	32	2	0	
35	2	32	0		
40	0	32			
100		32			

(a) Table 1; (b) Deplasmolysis; (c) Time min.;(d) Controls; (e) Test with heteroauxin.

Tabelle 2. (a)

17. 6. 49.

Zeit (b) min	Kontrolle (c)	Versuch mit Heteroauxin (d)			
		10^{-4}	10^{-5}	10^{-6}	10^{-7}
		Plasmolyse (e)			
25	71	35	80	100	100
30	80	50	90		
35	85	80	100		
50	100	100			
		Deplasmolyse (f)			
10	60	90	60		45
15					28
20	39	88	30		14
25	23	85	16		3
27		85			0
30	13	85	9	6	
35	11	84	4	0	
40	9	84	0		
45	4	84			
50	0	84			
100		84			
100% Deplasmolyse nach (g)	50 min		40 min	35 min	27 min

(a) Table 2; (b) Time min.; (c) Controls; (d) Test with heteroauxin; (e) Plasmolysis; (f) Deplasmolysis; (g) 100% deplamsolysis after...

Table 2 shows that these results may be readily obtained consistently in subsequent experiments, i.e., they are readily reproducible. In this test run, the HA had already been added to the plasmolytic agent beforehand. This held true with but few exceptions for all subsequent experiments. The time required for deplasmolysis has been some-

what shifted, while, in general, more time was required to achieve complete deplasmolysis, but here again, upon addition of 10^{-7} HA, the deplasmolysis time was only about half as long as in the controls. The inhibition of deplasmolysis at the beginning, which was experienced in the earlier experiments, is now absent. The reason for this was as mentioned above, that the HA had already been added to the plasmolytic agent beforehand. In later experiments it was also found that the initial inhibition only appeared when the plasmolytic agent was free of HA. Table 2 also contains data on plasmolysis. The protoplasts behaved alike up to 15 minutes in all solutions (excepting HA 10^{-4}). In all cases there was a gradual withdrawal of the protoplasts. Here, then, the transitory inhibition by the HA did not make its appearance. Very clearly distinguishable differences already make themselves apparent after 15 minutes. While 71% of the control cells have undergone plasmolysis, those treated with 10^{-6} and 10^{-7} HA have already all undergone plasmolysis. This promotion of the release of water from the cells thus corresponds fully to the intake of water which occurred during deplasmolysis.

The 10^{-5} concentration showed a considerably lower effect in prompting the fluid transfer in both sets of tests. HA 10^{-4} carries out an entirely different behavior both in plasmolysis and deplasmolysis. In the former one notes at the start a very powerful inhibition of the release of water, but this condition is only temporary, so that 100% plasmolysis is reached at the same time as the controls. There was also a powerful inhibitory effect during deplasmolysis, which, however continued, with the result that many of the protoplasts failed to reach the cell walls even after 100 minutes. During the first set of tests 32% of the cells remained permanently plasmolyzed and during the second set, 84%. Before going into these phenomena in greater detail, the results of the test runs will be given in keeping with the deHaan method of evaluation. Table 3 contains the Tk/Tv quotients for different HA concentrations. It is clearly shown that the deplasmolysis times with HA 10^{-7} are only half as long as those of the controls; the average value is 2.04. The quotient at 10^{-6} shifts to 1.50 and at 10^{-5} to 1.30. In this area, then, the acceleration gradually drops off. Concentrations of 10^{-3} still destroy all of the protoplasts; at 10^{-4} the same effect (+) does partially make its appearance, or the protoplasts were no longer able to return to their cell walls (-). Only in the tests carried out on 10 October did deplasmolysis take place entirely, accompanied by a powerful inhibition of the water

intake, which expressed itself in a quotient of 0.41. Other such tests in the same month were successful and are given in Table 20, below. The figures shown there are 0.62, 0.44, 0.70 and 0.53. The inhibitions can always be observed to take place, even when full deplasmolysis takes place. We were able to achieve this effect regularly only during the month of October with a concentration of 10^{-4}. Apparently, then, the Rhoeo protoplasts are more resistant to HA at this season than during the summer months.

Tabelle 3. *Heteroauxin.* (a)

	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
16. 6.49	+	–	1,25	1,50	2,08
17. 6.49	+	+	1,55	1,75	2,00
18. 6.49	+	–	1,14	1,33	2,00
20. 6.49	+	+	1,36	1,53	2,16
2. 8.49	+	–	1,25	1,43	1,85
13.10.49	+	0,41	1,31	1,68	2,16

(a) Table 3 Heteroauxin.

It follows from the above that physiological concentrations of 10^{-6} and, particularly, 10^{-7}, increase permeability to water. This effect drops off with increase in concentration and at 10^{-5} is only very slight. This behavior requires an explanation, as does the transitory inhibition which occurs at the start of deplasmolysis (Table 1), which was also observed by Koningsberger (1947). The phenomena which occur in the 10^{-6} HA solutions are at first sight particularly difficult to understand. It is our wish to attempt to give a unified explanation of the entire behavior as a whole in the portion of this paper which follows. To do so, we deem it best to start with the effects produced by concentrations of 10^{-3} and 10^{-4}.

HA 10^{-3} causes the protoplasts to disintegrate. It should be noted that the protoplasm of other plants is even more sensitive to HA. While with Rhoeo there is still some period of time in HA 10^{-3} before the protoplasts dissolve, all the cells of onion skin epidermis, Helodea leaves and Salvinia water leaves very quickly run out. Assuming that the manner in which the protoplasts are destroyed can enable one to draw certain conclusions about the mode of action of HA, we studied the process of destruction in Rhoeo and Allium in greater detail. To this end we first plasmolyzed the cells in Mannitol without any added HA. We then observed such a process taking place in the deplasmolysis which followed. After 10 to 15 minutes the surfaces of the free protoplasts start to show changes in both subjects.

With Allium, broad caps form, having a foamy structure (Fig. 1, 3a). The foam bubbles soon become larger and during the following 10 to 15 minutes they flow into one another (Fig. 1, 3b). The plasmalemma has therefore been dissolved, while the tonoplast still remains intact for a while. After a total period of 23 to 25 minutes, the tonoplast finally also dissolves and the cell sap pours out into the cell cavity. No foamy caps appear in Rhoeo. In their stead, small, light refracting droplets cluster about the outline of the free protoplasm (Fig. 2, 3a). During the following 5 to 10 minutes these droplets cluster about the outline of the free protoplasm (Fig. 2, 3b). After a total of 23 to 25 minutes there also occurs here a step-by-step dissolution like the one that takes place in Allium, with the droplets finally remaining free of the cell wall (Fig. 2, 3c).

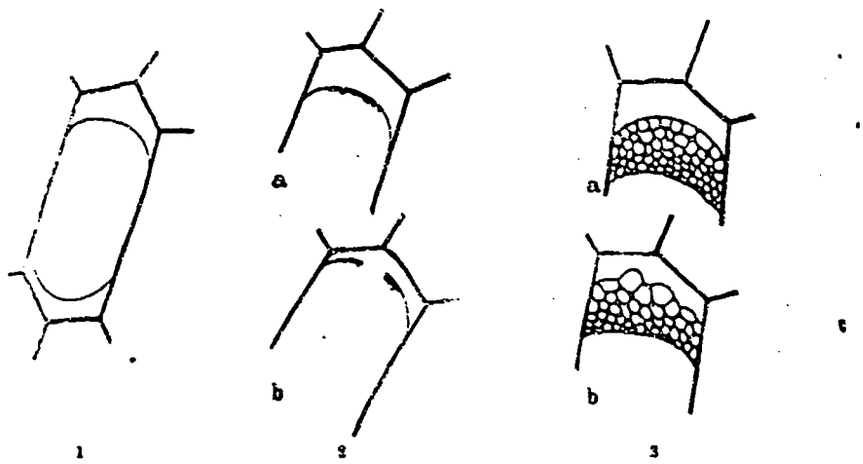

Fig. 1 - Allium epidermal cells. 1, Deplasmolysis in mannitol solution; 2a, b, the same with addition of parasorbic acid 10^{-2}; 3a. b. the same with addition of HA 10^{-3}.

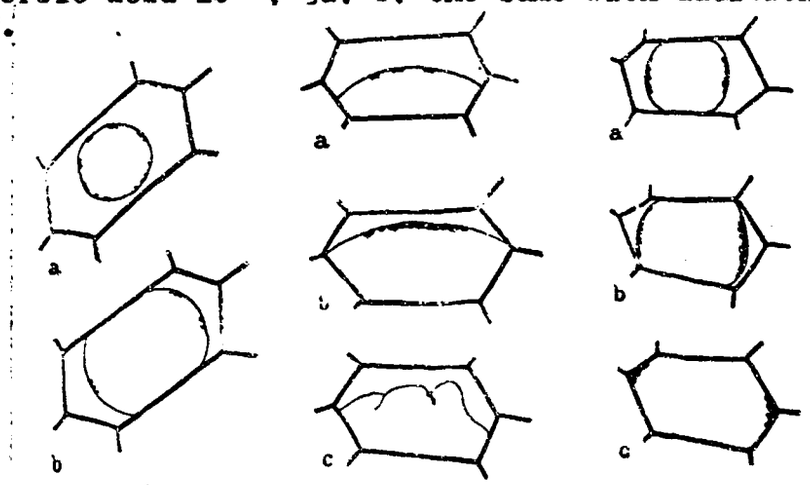

Fig. 2 - Rhoeo epidermal cells. 1a, b, progressive deplasmolysis in mannitol solution; 2a, b, c, the same with addition of parasorbic acid 10^{-2}; 3a, b, c, the same with addition of HA 10^{-3}.

The destruction of protoplasts herein described also takes place in HA 10^{-4} when the cells are in a state of heightened sensitivity. At the same time we found in many cases where no destruction took place that rather the deplasmolysis could no longer completely take place so that permanent plasmolysis had occurred. Two possible explanations can be found for this. One explanation is that of a large increase in viscosity. The protoplasm's resistance

to the entrance of water would then be overcome as long as the suction tension of the cell was still high. With swelling of the protoplasts the effective osmotic pressure would then decrease and the suction could finally no longer overcome the resistance of the protoplasm. This operates -- even if in a different way -- just as restrictingly as cell wall pressure. Another explanation would consist in the fact that in this case, too, one could already assume the existence of a certain degree of injury to the protoplasm. This would allow osmotic contents to be released during deplasmolysis and the halt in deplasmolysis would be attributable to the isotonicity already achieved with the external solution. In fact, one can achieve complete deplasmolysis if the deplasmolytic agent is further thinned. This fact alone, though, does not enable one to make any decision in favor of one or the other of the two possibilities. We believe, however, that the first of the two alternatives suggested is correct since we never saw any anthocyanin issue forth; this is all the more so because, apparently, an increase in viscosity and the destruction of the protoplasm are entirely different processes. The former is a reversible process, while the latter involves irreversible structural changes.

It is easy to understand that the protoplasts are not destroyed by the plasmolysis in the 10^{-4} solution. During plasmolysis, the tension of the protoplasm membrane decreases, while during deplasmolysis it increases, and the protoplasts subjected to tension would understandably more readily break down than those released from tension. Furthermore, the outwards flow of water associated with plasmolysis at first keeps the biocatalyzer away from contact with the protoplasm membrane, while inward flow, on the contrary, brings such biocatalyzers in contact with the membrane. This phenomenon should be borne in mind in all plasmolysis-deplasmolysis investigations of this kind.

It is more difficult to understand the behavior of the cells in the "physiological" concentrations of 10^{-5} to 10^{-7}. We see that the increase in permeability runs <u>contrary</u> to the concentration gradient. A concentration of 10^{-5} differs very little in its effect from the results obtained with controls, but it would be quite impossible to assume that HA in this concentration remains as good as ineffective.

The entire behavior becomes understandable at once if one attributes a double action to HA. It has recently

become increasingly clear that HA only promotes growth when it is present in intimate association with protoplasm. This is particularly clear from the works of Geiger and Sutter (1943) and Sutter (1944). Cucumber hypocotyls which are immersed in a 10^{-6} solution of HA will combine with only a limited portion of the substance offered them. The number of molecules which combine are insufficient by far to cover the entire surface area of the protoplasts. It can therefore be assumed that only specific sites of reaction are present in protoplasm, at which sites the substance forms a bond. We may now assume that only the HA which has formed a bond or combined with the protoplasm is able to increase water permeability, while the free HA works against the combined HA to decrease permeability and that too high a concentration of free HA ends in destroying the protoplasm. The amount of free HA in the 10^{-4} solution predominates to such an extent that it brings about a condition of powerful inhibition. With a concentration of 10^{-5} the inhibition becomes weaker and approaches the point where it almost equals the amount by which permeability is promoted, achieving an almost equal balance or a compensating effect; compared with controls, only a slight promotion or favoring of water intake exists at this point. With concentrations of 10^{-6} and 10^{-7}, the inhibitory effect finally decreases to such an extent that only the promotional effect remains, reaching its maximum level. Such an effect could be of a direct nature, but a large amount of data speaks for the fact that it is of an indirect kind. One of us (Guttenberg 1942) has put forth in numerous papers the theory that the effect of HA is not direct, but that it depends much more on the fact that it frees the auxin combined with the protoplasm by displacing it and thereby activates it. This process has already been carried out during the first interaction with protoplasm by means of a displacement process. The permeability promoting factor would no longer then be the combined HA, itself, but rather the auxin /See Note/ thus freed by this process. This theory could perhaps also explain the initial inhibition during deplasmolysis in 10^{-5} to 10^{-7} solutions during the first experiment (Table 1). The added HA is first free and must consequently have the effect of increasing viscosity. Only after several minutes when it has gone into combination does its second, promotional effect come to bear.

(/Note:/ Auxin is taken to mean both here and subsequently that growth factor which demonstrates itself to be acid resistant. There can hardly be any doubt that such an agent does exist since numerous authors have found that

plant extracts which were boiled with acids showed negative curvatures in the Avena test or oat coleoptile test and a corresponding behavior in other tests. Thus recently Avery, Berger, and White were able to extract both an acid resistant and a lye-resistant growth stimulant from cabbage varieties, both of which substances were found to be present partially in a free state and partially in combination. Whether or not these acid resistant growth factors represent auxin a and b described by Kögl or not will not be dealt with here; as long as these substances remain unavailable the question cannot be resolved one way or the other. When the manuscript of this paper was already finished, a paper by Reinert (1951) appeared in which HA was identified as the growth stimulant for oat coleoptiles. This agrees with earlier data by Wildman and Bonner (1948). In subsequent investigations we also discovered a growth factor in ether extracts of coleoptiles which is destroyed by acids and pea enzymes, thus corresponding to HA. It would seem, though, that the extracting agent is of great importance to the results obtained from such experiments. We will shortly report on tests in which we obtained from the same material in one case acid resistant growth factors by extraction with alcohol and in the other case lye-resistant growth factors by extraction with ether, wherein only the second of the two substances was destroyed by pea enzymes. If the acid-resistant enzymes have no connection with Kögl's auxin, then a new possibility presents itself, namely, that there are two modifications of HA, one which is acid-resistant and migrates to the lower leaf base side and the other of which is lye-resistant, being diffused to the opposite pole.)

Mention should also be made of several new papers which appeared during the course of our work. Above all the recent work of Koningsberger is significant. He worked with isolated Allium-protoplasts (onion skin mesophyll) and used glucose as a plasmolytic agent. He holds that it is important to isolate the cells, otherwise an absorption of the biocatalyzers by the cell membrane would keep these substances away from the plasmalemma. We could raise the objection that the solution is available in a sufficiently large surplus to render this possibility hardly worth considering. On the other hand there is always the danger that the protoplasts, in being isolated, will be damaged and, finally, that the use of sucha method will never make available any more than a few individual protoplasts for observation. Koningsberger, like ourselves, noted the existence of a powerful initial inhibition of deplasmolysis when he added HA 10^{-6} or 10^{-7}. Swelling started afterwards, but it occurred

more slowly than in the controls. In the end some of the protoplasts were smaller, but others were much larger than those of the controls. Koningberger, himself, spoke of his results as being of a preliminary nature. On the other hand, the number of cells which were tested by us was so large that we cannot have any doubt as to the correctness of our findings. In addition, the most important fact to Koningsberger was the proof that HA first affected the protoplasm and not the cell wall. In many respects his own thoughts agree with ours. Brauner and Hasman (1949) further developed the results of investigations carried out by Reinders (1942). They found that slices of potatoes and other tubers and turnips showed a clear increase in water intake when HA was added in concentrations of 10^{-5} to 10^{-6}. This fact was substantiated by Commoner et al (1943), Overbeek (1944) and Levitt (1948), in three papers which unfortunately are unavailable to us at the moment. These authors attempted to explain the water intake partially on the basis of an increase in osmotic substance associated with an increase in respiration. Lastly, Levitt looked upon an increase in the plasticity of the cell wall as a cause. In contrast, Brauner and Hasman found that HA 10^{-5} and 10^{-6} made it easier for water to enter the tissues, which is entirely in agreement with the above mentioned investigation by Pohl (1949).

2. Avena growth stimulant. In our earlier tests with Phaseolus joints, the diffusate from oat coleoptile tips produced the same effect as HA. We now used an extract made up in accordance with data provided by Moewus (1949) for our plasmolysis tests. 10 mm. were cut off from the tips of oat coleoptiles after they had reached a length of 4 to 5 cm; a hundred of these tips weighed about 0.7 grams. They were then ground together with 10 ml. of twice-distilled water and one drop of 10% acetic acid, using a mortar and pestle. The extract was kept at 4°C. in the dark for a 24-hour period. We then filtered the extract and 7 ml. were thus obtained. According to Moewus the filtrate thus made up has the same effectiveness in the cress root test as HA 10^{-6}. We further diluted our filtrate so that solutions corresponding to HA 10^{-7}, 10^{-8} and 10^{-9} were made ready. These concentrations are shown in Table 4.

Tabelle 4. *Avena-Wuchsstoff (cl)*

	10^{-7}	10^{-8}	10^{-9}
16. 1. 50	1,50	2,00	1,00
24. 1. 50	1,30	1,74	1,00
25. 1. 50	1,20	1,50	1,00

(a) Table 4 Avena Growth Factor

As is shown in Table 4, both the 10^{-7} and 10^{-8} concentrations had a favorable effect on deplasmolysis, while this effect disappeared in concentrations of 10^{-9}. The maximum effect was achieved at 10^{-8}. If one compares these findings with the HA tests (table 3), then one will see that the comparable concentrations which had previously been calculated as equivalent to 10^{-8} and 10^{-7} HA, are actually more nearly equivalent to 10^{-7} and 10^{-6}HA, respectively. Apparently our filtrate was more concentrated than that prepared by Moewus and it must have, in fact, corresponded to an HA concentration of 10^{-5}. The fact that coleoptile tip extracts do not always have the same concentration of growth factors was also brought out later on as is shown in Table 22. In this case the relationship between Tk/Tv at concentrations of 10^{-7} averaged 1.15; at 10^{-8}, though, it was 1.47. This corresponds roughly to the HA concentrations of 10^{-5} and 10^{-6} in Table 3, respectively.

Natural growth factors obtained directly from plants, therefore clearly accelerate the deplasmolysis rate when applied in physiologically effective dilutions, thereby increasing water permeability.

Also in the application of Avena growth factors we came across the fact that the effectiveness increases as the concentration diminishes. This relationship has already been known to exist for many biocatalyzers but to date it has not been subjected to sufficient analysis. Here, too, one comes closer to the truth if one assumes that the substance has an inhibiting effect along with its promoting effect. In order that it may carry out its promoting action, molecular bonds must apparently be present at specific sites in the protoplasm. As long as these sites are occupied, the excess of active material has an inhibiting effect

at some other site. We were never able to observe the presence of any absolute inhibition utilizing Avena growth factors. This though, would only be attributable to the fact that the high concentrations required to do so could not be produced.

3. Phenylacetic Acid. Among the well-known synthetic growth promoting agents, we unfortunately had only phenylacetic acid available to us. It is soluble in water in concentrations of 10^{-2}. As is shown in Table 5, diluting it to 10^{-5} clearly brings about an acceleration of deplasmolysis. At this point it reaches a maximum and its potency corresponds approximately to HA 10^{-6}. In further dilution, (10^{-6}, 10^{-7}) phenylacetic acid remains without any effect. It also seems to lose its effectiveness in concentrations of 10^{-4}, though it should again be assumed that here, too, two different processes are compensating for one another, namely one a growth promoting and the other a growth inhibiting action. Hence, the next step to 10^{-3} already shows a clear inhibition of water intake. In concentrations of 10^{-2} the protoplasts are already destroyed within two minutes. The cell sap becomes pink and runs out.

The behavior of the 10^{-3} concentration needs to be described in still fuller detail. There is already a difference compared with the controls even during plasmolysis. It is true that plasmolysis starts at the same time as it does in the controls, but it takes place at a faster rate, which did not occur with the other concentrations. Soon afterwards it comes to a stop and 15 to 20 minutes later the protoplasts dissolve, going through the same processes as described for HA 10^{-5}. In order to be able to study the deplasmolysis, we plasmolyzed the piece cut out from the leaf without any phenylacetic acid at first and placed it in the deplasmolyzing agent. Here, too, the majority of the protoplasts was destroyed, but in several cases we were able to observe an inhibition of deplasmolysis lasting 10 to 20 minutes. This inhibition is shown in Table 5.

Tabelle 5. *Phenylessigsäure.*(a)

	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
28. 8. 39	0,51	1,01	1,47	1,00	1,00
29. 8. 39	0,70	1,00	1,44	1,00	1,00
30. 8. 39	0,81	1,00	1,80	1,00	1,00

(a) Table 5 Phenylacetic Acid

Within the next 3 to 5 minutes, the protoplasts

expanded with great speed and they always burst upon impact with the cell wall. Apparently, then, after 10 to 20 minutes the same damage was achieved as occurred immediately upon use of the 10^{-2} HA solution.

In using the Went Avena test, no curvature was obtained in agar using 10^{-3} and 10^{-4} phenylacetic acid solutions, whereas with 10^{-5} to 10^{-7} solutions, negative curvatures appeared after $1\frac{1}{2}$ to 2 hours. The absence of curvatures at 10^{-3} and 10^{-4} may be pointed to as the results of compensatory effects. These concentrations should have led to a positive curvature in test plants free of growth factors, but since the coleoptiles, themselves, contained growth promoting substances, the effects could have canceled each other out. The fact that even concentrations of 10^{-6} and 10^{-7} still gave negative angles may be explained, perhaps, by the fact that, even though these concentrations were ineffective during plasmolysis, their application had the effect of summation instead of a counteracting effect. It is also possible, too, that the coleoptiles are more sensitive to phenylacetic acid than are the Rhoeo protoplasts. Further confirmation is given by the fact that maximum growth promotion in the Avena test was also found to occur at 10^{-5}.

4. Colchicine. The effects of treatment with colchicine resemble many of those induced by treatment with HA. Thus the appearance of club-shaped roots has already been known for a long time. According to Mairold (1943), colchicine has an inhibiting effect on the growth of lupine roots in concentrations of about 10^{-3} to 5×10^{-4}, while in greater dilution (10^{-5}) it has a growth-promoting action. More important for our study, though, is the fact that Mairold, in working with Allium and Spirogyra, discovered a shortening of plasmolysis time after preliminary treatment with 0.4% colchicine. Weissenböck (1950) noted a sharp reduction in plasmolysis and deplasmolysis times in the stomate mother cells of cotyledons treated by him with colchicine. He attributed this effect to a decrease in viscosity.

Tabelle 6. Colchicin. (a)

	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
24. 9. 49	0,80	1,50	1,50	1,80	1,00
26. 9. 49	0,80	1,16	1,16	1,40	1,00
27. 9. 49	0,80	1,40	1,43	1,66	1,02
28. 9. 49	0,81	1,33	1,40	1,60	1,00

(a) Table 6 Colchicine

As is shown by Table 6, colchicine affects water permeability of protoplasm just as growth factors do. Concentrations of 10^{-4} to 10^{-6} increasingly accelerate deplasmolysis, while 10^{-3} brings about a definite inhibition and 10^{-7} remains without any effect whatever. Here, too, then, two counteracting processes would seem to be present, one of which perhaps consists in the activation of auxins. Our findings therefore agree quite well with the results obtained by Mairold. Reese (1950) noted an optimum stimulation of longitudinal growth of hypocotyls (Lepidium, Petroselinum) in a concentration of 10^{-4}; on the contrary, colchicine 10^{-2} has an inhibiting effect, as does 10^{-3} to some extent on these subjects. Thus far his results agree both with those of Mairold and our own. Unlike Mairold, though, Reese also found that both low and high concentrations (0.01%) stimulated root growth; nor was he able to bring about any curvature with colchicine, neither by using the Avena test, nor in Helianthus seedlings. He concludes from this that colchicine could have a final result similar to that of growth-promoting agents, but that such an effect would be brought about in an entirely different way. According to his results one could not even consider the possibility of any activation of auxins on the part of colchicine. Only further investigations can finally decide whether his interpretation is correct or not.

5. Vitamin C. Raadts (1949) observed that Vitamin C in concentrations of 10^{-5} to 10^{-7} has a growth-promoting effect on coleoptiles; he attributes this to its activation of auxin. We tested the effect which this vitamin had on water permeability, first using a sea buckthorn berry or sallow thorn berry (Hippophaë rhamnoides) extract, which we prepared in accordance with instructions given by Moewus (1949). 15 grams of ripe berries were crushed with mortar and pestle in 15 ml. of distilled water together with clean sand. After removal by suction, 20 ml. of filtrate were obtained, representing the starting solution. Upon dilution in a 10:1 ratio, the concentrations shown in Table 7 were obtained. As one can see, the extract diluted to 10^{-1} used in the tests partially destroys the protoplasts (+) and partially prevents a complete return from plasmolysis (-). The protoplasts underwent marked changes. They showed abnormal contours and they behaved like those in HA 10^{-3} when being destroyed. The 10^{-2} concentration brings about a considerable delay in the return of the protoplasm, while deplasmolysis is, instead, promoted by 10^{-3} to 10^{-5} concentrations, reaching a maximum at 10^{-4}. The effect disappears with 10^{-6}.

Tabelle 7. *Stranddornbeerenextrakt.* (a)

	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
2. 10. 49	±	0,65	1,50	1,82	1,33	1,02

(a) Table 7 Sea Buckthorn Berry Extract

The high ascorbic acid content of sea buckthorn berries makes it probable that ascorbic acid is the agent responsible for the above described effect. On the strength of this we proceeded to work with pure vitamin C. As a starting solution we chose 0.1N ascorbic acid, since this solution had the same pH as the sea buckthorn berry extract. The starting solution thus contained a concentration of 1.76×10^{-2}; the 1/10 dilution of this solution was made up as usual, so the first step in dilution gave an ascorbic acid content of about 10^{-3} grams per ml.

This concentration destroyed most protoplasts after only 2 to 4 minutes; the cell sap ran out. 10^{-5} to 10^{-7} concentrations promoted deplasmolysis, the maximum being in the vicinity of 10^{-5}. 10^{-4} and 10^{-8} showed results similar to those observed in the controls (Table 8). One must again assume that two effects are taking place at 10^{-4}, one promoting and the other inhibiting, which compensate each other, while at 10^{-8} no effect takes place because of too great a dilution.

Tabelle 8. *Vitamin C.* (a)

	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
8. 10. 49	+	1,02	1,46	1,38	1,19	1,05
12. 10. 49	+	1,00	1,43	1,25	1,11	1,01
14. 10. 49	+	1,10	1,66	1,50	1,10	1,00

(a) Table 8 Vitamin C

If we compare the sea buckthorn berry extract with the pure vitamin C, then the extract concentrations of 10^{-4} and 10^{-5} would correspond approximately to vitamin C concentrations of 10^{-5} and 10^{-6}, respectively. The extract therefore works more weakly than the vitamin concentrations employed, which is also brought out by the fact that in concentrations of 10^{-1} of the former the protoplasts are not always destroyed. It is however also possible that the sea buckthorn berry extract contains an additional inhibiting

material, which appears as an antagonist to ascorbic acid and minimizes its deleterious effects. This would also explain the fact that 10^{-2} concentrations of the extract have an inhibiting effect, which is absent in the pure solution. Neither can the effect of using ascorbic acid be explained by fluctuations in pF, because the pH changes only very little over the range of the effective concentrations, 10^{-4} to 10^{-7}.

Our results compare well with the results of Raadts (1949), since the coleoptiles investigated by him were aided in their growth by 10^{-5} to 10^{-7} concentrations which induced an increase in water intake, whereas in our tests the effect finally dropped off. Raadts observed a growth promotional effect during only the first 2½ hours. After five hours an inhibitory effect set in which remained unchanged for a long time thereafter.

b) Growth Inhibitors.

1. Coumarin. Beldstra and Havinga (1943) found in tests carried out on cress seeds that coumarin is a specific growth inhibiting agent having blastocholin-type properties. Even in a concentration of 10^{-3} to 10^{-4} coumarin prevented these seeds entirely from germinating. About the same time Kuhn and Merchel (1943) showed that this substance surpresses germination of the pollen tubes of Antirrhinum majus and that it inhibits reproduction of certain bacteria and yeasts. Finally, Moewus (1949) found that coumarin also inhibits the longitudinal growth or elongation of cress roots within the range of concentrations between 10^{-3} and 10^{-6} grams per ml. With the aid of the paste method Moewus also demonstrated inhibition of oat coleoptiles; on applying concentrations of 10^{-3} the coleoptiles underwent positive nastic movements.

These results led us to test the effect of coumarin on water permeability with the aid of our method. We carried out the tests as before in our earlier tests, i.e., we added the coumarin to mannitol solutions in a series of different concentrations. Nothing more need be said about behavior of the specimens during plasmolysis since they reacted in the test solutions in the same way as in the control solutions. Upon deplasmolysis, however, a general permeability inhibiting action appeared as shown in Table 9. After 5 minutes the inhibition of water intake is very extensive. While in the control cells only half of the cells remain plasmolyzed, 80% to 90% of those in the coumarin

solution are still plasmolyzed. With further passage of time it is found that in coumarin 10^{-3} the great majority of protoplasts still have not reached the cell wall even after 100 minutes, so that one might well say that permanent plasmolysis had set in. Even in 10^{-4} concentrations it is a long time before all cells have been deplasmolyzed. While all control cells have been deplasmolyzed after 37 minutes, 40% of the cells treated with coumarin still remain plasmolyzed. The water intake occurs somewhat faster in 10^{-5} coumarin solution. As can be seen, the range of concentrations over which water permeability decreases compares approximately with that noted by the other authors mentioned earlier in tests carried out by them.

20. 6. 49. Tabelle 9. *Deplasmolyse.*

Zeit min (a)	Kontrolle (c)	Versuch mit Cumarin (d)		
		10^{-3}	10^{-4}	10^{-5}
5	51	90	90	80
10	39	88	82	68
15	29	88	70	46
20	25	86	65	31
25	22	85	55	26
30	10	71	45	15
37	0	71	40	7
40		71	15	0
45		71	8	
		70	0	
		70		
100% Deplasmolyse nach (e)	37 min	—	50 min	40 min

(a) Table 9 Deplasmolysis; (b) Time min. (c) Controls (d) Test with coumarin (e) 100% deplasmolysis after..

Further tests wherein the Tk/Tv ration (deplasmolysis time of controls divided by deplasmolysis time of test subjects) was tested for gave the results shown in Table 10. The delay in deplasmolysis in coumarin 10^{-3} was very large here. Nevertheless, all of the protoplasts finally returned to their cell walls except in the tests carried out on 10 June. Here, too, the inhibition in 10^{-4} was much more marked than in 10^{-5} and in certain cases there was no inhibition at all in 10^{-5}; it was absent in all cases on using 10^{-6} concentraions.

Tabelle 10. *Cumarin.* (a)

	10^{-3}	10^{-4}	10^{-5}	10^{-6}
21. 3. 49	0,25	0,60	0,91	1,00
26. 3. 49	0,23	0,66	1,00	1,00
27. 3. 49	0,16	0,55	0,[illegible]	1,00
29. 3. 49	0,33	0,54	1,00	1,00
20. 6. 49	—	0,76	0,96	1,00

(a) Table 10 Coumarin

A very marked difference from HA and the other growth promoting substances consists in the fact that no reversal of effect takes place at any concentration and that the inhibitory effect increases with increasing concentration. Coumarin therefore always carries out an inhibiting effect. It would seem that this substance brings about an increase in viscosity the degree of which depends on the concentration which, in the end, upon being too greatly diluted, fails to take effect. If permament plasmolysis set in during the first run of tests in the 10^{-3} solution, this can readily be explained in the same way as the permanent plasmolysis on the occasion of the tests in HA 10^{-4}.

If coumarin inhibits elongation, then, it can be understood that the intake of water into the cells, which is necessary of this process, is inhibited. On application to one side as in the coleoptile experiment by Moewus, a positive nastic movement must take place because the side thus treated takes up less water into its cells than the untreated side. Coumarin undoubtedly operates directly on the protoplasm and not by way of any deactivation of auxins. It represents an independently acting antagonist as is brought out in the compensation tests mentioned later in this paper. Upon examining the results of Moewus one further finds that coumarin 10^{-3} inhibits the growth of both roots and coleoptiles. On the other hand, in the case of auxin, it is known that concentrations which inhibit root growth (approx. 10^{-6} HA), promote the growth of coleoptiles. Our observation that coumarin inhibits in all concentrations renders the results of Moewus understandable. If auxin were deactivated by coumarin, then one would expect to find an acceleration of root growth; the fact that this does not occur is a further proof of the direct action exercised by this substance.

2. Tomato Extract. According to Larsen (1939)

tomatoes contain various germination and growth inhibiting substances. This led us to investigate whether these substances modify deplasmolysis rates. Larsen had prepared ether extracts from tomato juice, evaporating it until almost dry. The resulting dark-red, greasy feeling mass was taken up by him in lanolin and he tested this paste on oat coleoptiles; using this he obtained positive nastic movements or curvatures. We needed a water solution for our plasmolysis investigations, though. We therefore triturated 100 grams of ripe tomatoes in 50 ml. of distilled water and drew the extract off through a suction filter 24 hours later. The filtrate thus obtained was concentrated to 10 ml. and was used as our starting solution. The 10^{-1} dilution (Table 11) brought about a very clear inhibition of water intake. With a 10^{-2} dilution there was already a sharp reduction in its action since an inhibiting action was observed to take place in only one test run; 10^{-3} had no effect. The tomato extract therefore contains a water permeability reducing inhibitor which is apparently effective only in relatively high concentration.

Tabelle 11. *Tomatenextrakt.* (1)

	10^{-1}	10^{-2}	10^{-3}
4. 9. 49	0,33	0,66	1,00
6. 9. 49	0,40	1,00	1,00
7. 9. 49	0,50	1,00	1,00

(a) Table 11 Tomato Extract

According to Akkerman and Veldstra (1947) tomatoes contain inhibiting ferulic and caffeic acids. We were able to confirm this, but we discovered further non-acid inhibitors, which, unlike ferulic and caffeic acids, inhibit the development of staphylococci.

3. 4-hydroxycoumarin. This chemical, so kindly made available to us by Heidelberg, works as an inhibitor in the cress root test according to Moewus (1949). Our starting solution made with warm water represented a dilution of 10^{-3}. This concentration completely inhibits deplasmolysis (permanent plasmolysis) or destroys the protoplasts. The 10^{-4} and 10^{-5} dilutions slow down the water intake with decreasing effectiveness and the effect disappears with 10^{-6} (Table 12). The inhibiting effect of this substance during the cress root test takes place over the same range of concentrations according to Moewus.

Tabelle 12. *Benztetronsäure.*(a)

	10^{-3}	10^{-4}	10^{-5}	10^{-6}
22. 11. 49	∓	0,40	0,80	1,00
23. 11. 49	∓	0,33	0,70	0,90
24. 11. 49	—	0,35	0,71	0,98

(a) Table 12 4-hydroxycoumarin

4. Ferulic Acid. We also obtained the ferulic acid from Heidelberg. It is found in Ferula Asafoetida and in various conifers, as well as in other substances. We prepared a 10^{-3} starting solution by warming it in water. This concentration (Table 13) shows a very powerful inhibiting effect, while 10^{-4} has a weaker inhibiting effect. The effect disappears at 10^{-5} and 10^{-6}. Ferulic acid therefore demonstrates itself to be a typical inhibitor in deplasmolysis tests.

Tabelle 13. *Ferulasäure.*(a)

	10^{-3}	10^{-4}	10^{-5}	10^{-6}
20. 11. 49	0,[illegible]	0,69	1,00	1,00
23. 11. 49	0,50	0,76	1,00	1,00
25. 11. 49	0,47	0,77	1,00	1,00

(a) Table 13 Ferulic Acid

5. Parasorbic acid. Parasorbic acid is taken from rowanberries and has an inhibiting affect on the germination of the seeds. In addition it also inhibits elongation or longitudinal growth. Applied as a paste in a concentration of 10^{-3} it induces positive nastic movements in oat coleoptiles according to Moewus (1949).

To prepare a stock solution, one part parasorbic acid was dissolved in 10 parts water (= 10^{-1}). We obtained the acid through the kindness of Dr. Moewus from the chemical stock of the Max Planck Institute for Medical Research in Heidelberg and we wish again at this point to express our sincere thanks to him for his assistance.

As can be seen from Table 14, parasorbic acid also works as a typical inhibiting agent in deplasmolysis tests. In concentrations of 10^{-2} it destroys the protoplasts even during plasmolysis after 20 to 25 minutes. First of all small bubbles formed on the sharply contoured protoplast

edge (Fig. 2, 2). After 20 to 25 minutes have elapsed the protoplast suddenly bursts. In order to carry out studies of deplasmolysis, then, the leaf cuttings had first to be plasmolyzed without the addition of any parasorbic acid. On transferring such leaf sections into the deplasmolysis medium containing 10^{-2} parasorbic acid, the protoplasts burst in any case after from 1 to 10 minutes.

Tabelle 14. *Parasorbinsäure.*(a)

	10^{-1}	10^{-2}	10^{-3}	10^{-4}
11.11.49	+	0,26	0,75	1,00
14.11.49	+	0,31	0,78	0,98
17.11.49	+	0,31	0,76	0,99

(a) Table 14 Parasorbic Acid

This destruction was investigated in detail in Allium and Rhoeo cells and was drawn (Fig. 1, 2 and 2, 2). Both subjects begin to gather the bubbles mentioned above along the edge of the protoplast 2 to 3 minutes after the start of deplasmolysis. Compared with the small, round microsomes visible during normal deplasmolysis, these are very much enlarged, increased in number and flattened in shape. The formation of caps noted in 10^{-3} HA with Allium is entirely absent. In both subjects the bursting takes place at a limited site of the protoplast, in that an opening appears at the vertex of the protoplasm swelling where the cell sap runs out; the tonoplast then slowly collapses upon itself. The process of destruction is thus entirely different from that undergone in HA. One gains the impression that all in all we are not dealing here with any dissolution of the protoplasm as occurs in HA, but rather with an increase in viscosity which greatly decreases the elastic flexibility of the protoplasm membrane. As already mentioned, the destruction occurs much more rapidly during deplasmolysis than during plasmolysis. This can readily be explained by the fact that entering water increases the tension of the protoplasts, while the removal of water into the plasmolysis medium decreases the tension.

The 10^{-3} and 10^{-4} concentrations have a very powerful inhibiting action, while 10^{-5} remains almost without any effect at all. These results correspond well with those obtained by Moewus (1949) on the effect of parasorbic acid on the growth of cress roots. There, too, with the 10^{-5} concentration the effect disappears; on the other hand 10^{-2} brings about a complete inhibition of growth.

Further tests were carried out with mountain ash berry extract. This extract was prepared in the same way as the sea buckthorn extract and gave the results shown in Table 15.

Tabelle 15. *Ebereschenextrakt.*

	10^{-1}	10^{-2}	10^{-3}	10^{-4}
27. 9. 49	—	0,61	0,72	1,00
30. 9. 49	—	0,63	0,70	1,00
1. 10. 49	—	0,62	0,71	1,00

(a) Table 15 Mountain Ash Berry Extract

In dilutions of 10^{-1} we obtained in all cases permanent plasmolysis, but never destruction of the protoplasm. Presumably here, too, additional substances present in the extract work in antagonism and hence protect the protoplasts as in the case of the sea buckthorn extract. In comparing Table 14 with Table 15, one should note the fact that in the former the concentrations are given in grams per milliliter, while the figures in the latter represent only the dilution of the juices; the figures, therefore, cannot be used for direct comparisons.

6. Ethylene. As is known from numerous investigations, ethylene exercises highly diversified effects on the growth and development of plants. Investigations of germination and gemmation (seeds, leaf buds, potatoes, etc:) with ethylene were observed producing for the most part a growth stimulating effect, but under certain circumstances, an inhibiting effect was produced, depending on concentration and duration of action. Elongation on the other hand was always more or less inhibited, but the degree of inhibition depended less on the dosage of ethylene applied than on the plant's sensitivity to this substance. Thus, for example, the budding shoots of legumens and crucifers are very powerfully inhibited, while Helianthus hypocotyls are less inhibited and coleoptiles least of all. Ethylene's effect is undoubtedly the result of a complex process also because among other things it very powerfully affects respiration. Von Guttenberg and Steinmetz (1947) recently found that ethylene deactivates auxin (but not HA), since it brings about an irreversible blocking of this substance. Earlier Von Guttenberg had expressed the opinion (1936) that even the suppression of geotropism occurring in the so-called horizontal nutation depends on a loss of auxin. The striking thickening of the

bud aprouts would be explained if one assumed that inhibitors bonded locally up to that time would be liberated by increased permeability. In fact, the amount of inhibitors contained in those sprouts which react sensitively to ethylene (for example legumens) is great, while it is very small in the coleoptiles which react hardly at all, while in Helianthus it is intermediate in amount. It seemed to us that an investigation of the question of water permeability might aid in further clarifying the question of the effect of ethylene.

The starting solution was prepared in accordance with Ruge's instructions (1947) as follows: Coal gas containing ethylene was slowly fed through 100 ml. of tap water at 17°C. for 30 minutes. This solution was considered as being saturated and accordingly it contained 0.0158 grams of ethylene according to Landolt-Börnstein. Concentrations of from 10^{-1} to 10^{-6} were then prepared by further dilutions. According to Ruge, ethylene is the only physiologically active component of coal gas. The concentrations shown in Table 16 are those corresponding directly with those of Ruge. In reality our 10^{-1} solution corresponds to an absolute concentration of 1.58×10^{-5} grams per milliliter.

Tabelle 16. *Äthylen.* (?)

	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
8.10.49	1,50	1,87	2,14	2,08	1,00
10.10.49	1,66	1,85	2,15	2,08	1,10
11.10.49	1,52	2,04	2,20	2,05	1,00

(a) Table 16 Ethylene

As is shown in Table 16, ethylene has the effect of increasing permeability in all the concentrations tested, with maximum effectiveness occurring in the neighborhood of 10^{-3} to 10^{-4}. Only on reaching a concentration of 10^{-5} (= 10^{-9} grams per milliliter) does the effect disappear. It is important that 10^{-1} also still favors the intake of water and that at no place does any reversal into an actual inhibition take place. The fact that ruge observed an acceleration of germination at this concentration, even if only slight, agrees well with our findings. In his tests the concentrations between 10^{-3} and 10^{-6} caused a considerable increase in speed of germination, but had less effect on increasing the power to germinate. He, therefore, looks on ethylene not so much as a germination inducing agent as

a germination accelerating agent. We now presume that the acceleration which was observed was brought about by an increase in water permeability.

Since we also were unable to bring about any inhibition using ethylene saturated water, we tested to see whether it would occur if one prolonged the duration of the effect. We therefore left leaf cuttings in ethylene saturated water for up to 70 hours. Subsequent plasmolysis and deplasmolysis though resulted in a deplasmolysis time which was still always shorter than that of the controls. Ethylene's effect on protoplasm is therefore undoubtedly the result of an entirely specific activity. One can hardly conceive here of any liberation of auxin as we had assumed for HA; ethylene rather blocks auxin as stated above and inhibits growth. The increase in permeability mentioned here could, however, lead to an unregulated dissemination of inhibitors and in this way bring about the striking growth inhibition. Tests are underway to clarify this question.

Our ethylene experiments are also of interest because of the fact that they teach that not every increase in permeability is neccessarily responded to by an acceleration of growth. This corroborates the assumption that also in the case of growth promoting substances the increase in water permeability is not the only observable effect, but instead only the first of these, in the process of elongation. The correctness of this point of view is also supported by the combined experiment using ethylene + HA, which will be dealt with further on.

c) Other Biocatalyzers

Investigations were made on other biocatalyzers due to their general, overall importance, specifically, penicillin and the weed-killer or herbicide, 2, 4-dichlorphenoxyacetic acid, to determine their effect on protoplasma permeability.

1. Penicillin. Penicillin, in its calcium salt form was dissolved in water to give a solution containing 50,000 units per ml. None of the dilutions tested from 10^{-1} to 10^{-6} showed any effect on deplasmolysis time. Thus penicillin does not affect the water intake of the protoplasts. The mechanism of action of this substance must then be of a kind entirely different from any of the hitherto described materials. Growth inhibition produced in corn

roots by Bein, Signer and Schopfer (1947) using penicillin was not attributed by the authors to the penicillin, but rather to HA, which accompanied the penicillin as a metabolic end product of the fungus.

2. 2, 4-dichlorphenoxyacetic Acid Sodium Salt. The starting solution containing 10^{-3} was prepared by warming sodium 2, 4-dichlorphenoxyacetate in distilled water. The low water-solubility of the substance did not permit us to prepare any higher concentrations. It showed no effect on the deplasmolysis of Rhoeo protoplasts over the range of concentrations from 10^{-3} to 10^{-6}. Since the destructive effects of the sodium salt of 2, 4-dichlorphenoxacetic acid were particularly noted occurring with crucifer weeds, while monocotyledons remained more or less spared, it could be thought that Rhoeo did not represent a suitable subject for carrying out the tests. We therefore used epidermal tissues from red cabbage for further tests as they can readily be used for deplasmolysis experiments. The results were again negative, though, and we noted only a change in color of the red cabbage cuttings from red-blue to blue-violet. According to the results of foreign investigators, which were only available in book reviews, the sodium salt of 2, 4-dichlorphenoxyacetic acid carries out its effect inside the cells, for example, by mobilizing carbohydrates or in nitrogen metabolism. These effects could be independent of permeability. Also the sodium salts of 2, 4, 5-trichlorphenoxyacetic and skatosulfonic acids showed themselves to be ineffective in our tests.

d) Potassium and Calcium

To further supplement our investigations, we also studied the effect of one monovalent and one bivalent cation on the water-permeability of our test subject. This was done in particular because we wished to utilize cations as antagonists to various active agents. Starting solutions used were N KCl and N $CaCl_2$, both of which were then diluted in 1:10 steps. A 10^{-1} solution therefore corresponded to a 0.1 N solution.

Calcium (Table 17) demonstrated the expected inhibitory action only in concentrations of 10^{-2} and 10^{-3}, the effect being only weakly amplified in the second case. At a concentration of 10^{-4} a reversal takes place in that there is a weak, but distinct stimulation. 10^{-4} was comparable with the results in the controls.

Tabelle 17. *Calciumchlorid.* (?)

	10^{-1}	10^{-2}	10^{-3}	10^{-4}
30. 8. 49	1,14	0,88	0,80	1,00
1. 9. 49	1,14	0,88	0,80	1,00
5. 9. 49	1,21	0,83	0,71	1,00
6. 9. 49	1,12	0,81	0,69	1,00
8. 9. 49	1,25	0,90	0,80	1,00

(a) Table 17 Calcium Chloride

Using potassium (Table 18), one observes an acceleration of the water intake from 10^{-3} up to 10^{-6}, where it reaches a peak, suddenly ceasing altogether at 10^{-7}. With potassium no reversal of effect takes place in stronger concentrations.

Tabelle 18. *Kaliumchlorid.* (?)

	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
10. 9. 49	1,00	1,00	1,11	1,33		1,66	1,00
11. 9. 49	1,00	1,00	1,10	1,11	1,25	1,66	1,00
13. 9. 49	1,00	1,00	1,10	1,22	1,38	1,83	1,00
15. 9. 49	1,00	1,00	1,11	1,11	1,42	1,66	1,00
17. 9. 49	1,00	1,00	1,02	1,20	1,54	1,80	1,00

(a) Table 18 Potassium Chloride

The results of our tests agree well in principle with those obtained by De Haan (1935). No direct comparison can be made because De Haan worked with calcium and potassium nitrates instead of chlorides, since he also used Allium Cepa as his test subject and sugar-cane as his plasmolysis medium. The inhibition which he found induced by Ca^{++} ions in the 10^{-3} concentration was somewhat less than ours, while his K^{+} ions already carried out a promoting action in concentrations of 10^{-1}. De Haan also mentions the fact that in certain conditions the salt concentration can be determining factor as to whether the solution will have a swelling or a shrinking effect. We then investigated whether potassium's permeability heightening effect would lead to curvatures or nastic movements in Went's Avena test. In reality, oat coleoptiles were observed undergoing negative nastic movements when we used 10^{-5} and 10^{-6} KCl in agar; in the latter of the two cases the effect was larger, which agrees with the results of our deplasmolysis tests. With 10^{-6} the curvature started

to take place after an hour, while with 10^{-5} it did not become apparent until after two hours had passed.

The fact that alkali salts can promote growth on one hand and inhibit it on the other hand has already been demonstrated by Borris (1937, 1939). Spergula arvensis and Silene coeli-rosa hypocotyls underwent in some cases more than 100% growth acceleration in K and Na salt solutions, while $CaCl_2$ inhibited elongation.

e) Tests Using Various Agents in Combination

The following portion of this paper reports the results of experiments carried out with various agents in combination. As we already noted, they were carried out in the hope that the results would help give a clearer insight into the mechanism of action of the various substances.

1. Potassium + Calcium. First of all we tested KCl and $CaCl_2$ individually again in order to obtain exactly comparable figures. KCl in a concentration of 10^{-6} again promoted maximum water intake with the quotients D = 1.65; $CaCl_2$ in a concentration of 10^{-3} gave the most powerful inhibitory effect with D = 0.50. We then mixed equal quantities of these two solutions and added the mixture to the mannitol solutions.

According to the individual results, one would have expected that there would still have been an inhibition; this would follow because the Ca had inhibited considerably more than K had promoted. If we select as an example a deplasmolysis time for the controls of 20 minutes, then this time would have been 12 minutes for K (20:12=1.66), and for Ca 40 minutes (20:40=0.5). In the first case an acceleration of 8 minutes occurred and in the second a delay of 20 minutes. Thus on being mixed together a delay of 12 minutes would result, whereas (Table 19) complete compensation took place instead. This is all the more striking since the K was administered in a much more diluted form than the Ca. This predominance of the promoting effect will be further dealt with and considered more in detail in connection with other tests of various agents in combination.

Tabelle 19. *KCl 10⁻⁶ + CaCl₂10⁻³.*(a)

	KCl 10^{-6}	$CaCl_2$ 10^{-3}	KCl 10^{-6} + $CaCl_2$ 10^{-3}
1.10.49	1,60	0,50	1,00
3.10.49	1,66	0,52	1,00
6.10.49	1,60	0,47	1,14
7.10.49	1,71	0,52	1,03

(a) Table 19 KCl 10^{-6} + $CaCl_2$ 10^{-3}

The physiological antagonism between the ions of alkali salts and alkaline earth salts has been extensively investigated in model tests (Höber 1924 and accompanying bibliography). Here, too, it was found that the effects of salts in tests on mixtures did not simply add to one another, but that rather specific and different concentrations of both substances cancel each other out in their effectiveness, for example, in the swelling of gelatin or on the surface tension of lecithin sols.

2. Potassium + Heteroauxin. We again tested both substances individually and obtained a quotient D = 1.76 with 10^{-6} HCl. We used heteroauxin as an antagonist in the inhibiting concentration of 10^{-4}, which at this time of year, in October, does not destroy protoplasts, but gives an average inhibition of 0.57. Here, too, then, inhibition was considerably more powerful than growth promotion. On applying them simultaneously, extensive compensation took place; the average quotient was 1.12 (Table 20). The promotional effect of potassium therefore counteracted the more powerful inhibiting effect of HA more than was to be expected.

Tabelle 20. *KCl 10⁻⁶ und Heteroauxin 10⁻⁴.*(a)

	KCl 10^{-6}	H.A. 10^{-4}	KCl 10^{-6} + H.A. 10^{-4}
15.10.49	1,70	0,62	1,20
18.10.49	2,00	0,44	1,00
19.10.49	1,75	0,70	1,10
21.10.49	1,60	0,53	1,18

(a) Table 20 KCl 10^{-6} and Heteroauxin 10^{-4}

In principle, this is the same result as we experienced with the K + Ca combination. In the absence of more detailed knowledge of the structure of the protoplasm, it is impossible to give any certain explanation of the

phenomenon. We would like to add to our observations those of Geiger-Sutter (1943) and Sutter (1944) mentioned previously, according to which the HA in greatly diluted solutions forms a bond at some specific reaction site in the protoplasm and only then does it operate to promote water intake. We have in addition assumed, though, that free HA has a harmful effect so that the protoplasm's permeability is forfeited. If this free HA and the K were to carry out the actions at different sites, then one could expect a simple summation of the effects to take place; then HA at its sites would further inhibit, and in the same way K at its sites would further promote water intake. But since no summation occurs, but rather the K almost compensates for the inhibiting effect of the HA, one may assume that they both operate at the same site and mutually influence one another in their operation. One may well conclude from the overall results that applications of potassium counteract the harmful effects of high concentrations of HA.

3. Coumarin + Heteroauxin. 10^{-7} HA gave a very moderate acceleration D = 1.25 (Table 21) in the individual tests, with only one exception. The tests were carried out in March, so the low figure is presumably conditioned by the season of the year. 10^{-4} coumarin, on the other hand, gave the particularly strong inhibition of D = 0.44. The fact that in combination the inhibiting effect should predominate should be less of a surprise, then, than the fact that it predominated so little. Here again, then, we find that the promoting effect gets the better of the inhibiting effect. On the whole, then, one finds as a result of the tests that coumarin and HA act as antagonists and one can again assume that they do not carry out their actions independently of one another at different sites, but that they operate at the same site.

Tabelle 21. *Cumarin 10^{-4} und Heteroauxin 10^{-7}.* (a)

	H.A. 10^{-7}	Cum. 10^{-4}	Cum. 10^{-4} + H.A. 10^{-7}
22. 3. 49	1,28	0,42	0,90
27. 3. 49	1,25	0,50	1,00
28. 3. 49	1,75	0,40	0,90
29. 3. 49	1,25	0,44	0,80

(a) Table 21 Coumarin 10^{-4} and Heteroauxin 10^{-7}

Moewus (1949) also discovered an antagonism between HA and coumarin in the cress root test. He first investi-

gated the root growth in separate solutions and then in combined solutions and compared the results with the growth of controls in pure water. His test runs on 8 May 1947, for example, show that coumarin 0.5 x 10^{-5} (inhibition - 20%) together with HA 0.5 x 10^{-9} (promotion + 3%) mixed together gave a growth inhibition of -15%. This result almost corresponds to what one would have calculated. No such simple summation occurs in any of our investigations, as already mentioned. Due to the complete difference in methods, this difference in results should be no surprise. We tested only the primary effect of the biocatalyzers on isolated cells, while Moewus studied the growth process of entire organs.

4. Coumarin + Avena Growth Factor. For this experiment we used the earlier described oat-coleoptile extract; the degrees of dilution should also be understood in the same way as in the earlier Avena tests. We tested two concentrations, namely, 10^{-7} having an effect D = 1.15 and 10^{-8} with D = 1.47. The coumarin solution gave an inhibition of D = 0.58; the latter was thus considerably more powerful than the former.

The combination of coumarin with the less powerfully effective 10^{-7} concentration (Table 22) resulted, accordingly, in a distinct inhibition. As against this we found that on combining the coumarin with 10^{-8} extract this was no longer the case; rather a complete compensatory action took place. We therefore again find that there is no simple summation of the effect, but that rather the growth-promoting substance prevails. Thus that which we had carried out earlier was valid here, too.

Tabelle 22. *Avena-Wuchsstoff (A) 10^{-7}, 10^{-8} + Cumarin 10^{-4}.*(a)

	A. 10^{-7}	A. 10^{-8}	Cum. 10^{-4}	A. 10^{-7} + Cum. 10^{-4}	A. 10^{-8} + Cum. 10^{-4}
28. 1. 50	1,11	1,42	0,55	0,77	1,02
31. 1. 50	1,20	1,56	0,55	0,77	1,00
1. 2. 50		1,43	0,64		1,03

(a) Table 22 Avena Growth Factor (A) 10^{-7}, 10^{-8} + Coumarin 10^{-4}

5. Ethylene + Heteroauxin. In an earlier investigation (Guttenberg and Steinmetz, 1947), we found that ethylene deactivates the plant's own auxin by a kind of blocking effect, while heteroauxin or HA remains unaffected by it. It was therefore considered to be of interest to

combine these two substances and test them; in so doing HA was tested once in the 10^{-4} inhibiting concentration and once in the 10^{-7} promoting concentration. Our earlier results enabled us to conclude that the inhibition occurring in higher concentrations is a purely HA effect, but that the promotional effect is attributable to activated auxin; if the auxin is blocked by ethylene, then its effect will be absent.

We therefore first combined 10^{-3} ethylene having the powerful promotional effect 2.20 with 10^{-4} HA, having a Tk/Tv ratio of 0.57 and, therefore, highly inhibitory (Table 23). The figure obtained by mixing them was 1.10; this weak promotion corresponds approximately to the relationship between the effects of the substances which were used. Ethylene thereby completely overcomes the inhibition brought about by 10^{-4} HA. Here, then, is a case in which one can assume that each of the two substances carries out its function at a different site or works in an entirely different manner; in such a case, then, as previously mentioned, one would expect a simple summation of the effects.

Tabelle 23. *Äthylen 10^{-3} + Heteroauxin 10^{-4}.* (a)

	A. 10^{-3}	H.A. 10^{-4}	A. 10^{-3} + H.A. 10^{-4}
12. 10. 40	2,20	0,57	1,10

(a) Table 23 Ethylene 10^{-3} + Heteroauxin 10^{-4}

We then combined the promoting HA concentration, 10^{-7}, which gave a quotient of 1.91 in this case with 10^{-3} ethylene, whose quotient was 2.17. A summation of both these effects would have brought about an extraordinary acceleration of the water intake. In reality, though, it proved considerably less, relatively, being 2.62 (Table 24). This is in complete agreement with our interpretation that the promoting action of HA depends on auxin activation. Such an activation could only have partially taken place, here, since, as pointed out above, the auxin is largely deactivated by ethylene.

Tabelle 24. *Äthylen 10^{-3} + Heteroauxin 10^{-7}.* (a)

	A. 10^{-3}	H.A. 10^{-7}	A. 10^{-3} + H.A. 10^{-7}
13. 10. 40	2,17	1,01	2,62

(a) Table 24 Ethylene 10^{-3} + Heteroauxin 10^{-7}

Borris (1943) applied KCl in a 0.02 mol solution to Agrostemma Githago germ buds in a culture substrate and subjected them simultaneously to an ethylene atmosphere. The inhibition in hypocotyl growth induced by the ethylene was greatly reduced by the effect of the potassium. There was powerful relative promotion of growth, which actually remained less than the absolute amount of growth in plants not subject to the gas when potassium is added. From this one can also conclude that the effects of ethylene and potassium are of entirely different natures. To this extent the tests in combination also correspond to the behavior observed in plasmolysis tests when HA is added to solutions of ethylene in water. Borris also studied the behavior of hypocotyls in ethylene after the cotyledons had been removed. In this case the inhibiting effect of the gas was sharply reduced. Entirely similar results were obtained by Borgström (1939) with other dicotyledonous germ buds. This behavior would in our opinion be explainable in the following manner: The cotyledons supply both growth-promoting and growth-inhibiting substances. Ethylene decreases the effect of the former, while the latter remain unaffected. They therefore flow throughout the entire plant and into the hypocotyl from the cotyledons, while if the cotyledons are removed they will be absent.

SUMMARY OF RESULTS

The task of the investigation was to study the effects which various biocatalyzers have on the water permeability of protoplasm. Violet epidermal cells from the underleaf of Rhoeo were used as the subjects of our experiments. The acceleration or inhibition of water intake was measured with the aid of the deplasmolysis time method. We used mannitol as a plasmolysis medium, as the protoplasts are impermeable to this substance. In most cases a 0.3 molar solution was used for plasmolysis and a 0.2 molar solution for deplasmolysis. The various active substances to be tested were added to these solutions; their osmotic pressures were unaffected, since the concentrations of biocatalyzers added were so small. A paper to follow will show that all effects observed were due not to any changes in pH, but were, instead, of a specific nature.

First of all substances which promote longitudinal growth or elongation were tested, above all heteroauxin, oat coleoptile extract and phenylacetic acid, as well as colchicine and vitamin C. HA in concentrations of 10^{-5} to

10^{-7} shortens the deplasmolysis time with increased dilution so that at 10^{-7} the deplasmolysis occurs in only about half the time as in controls. Over this range of concentrations, then, HA increases water permeability. A sudden reversal of effect takes place at 10^{-4} and a very powerful inhibition occurs, which is shown by a prolongation of deplasmolysis time to about twice that of controls (Fig. 3). Many cells now show permanent plasmolysis as a result of damage, while others are destroyed. This destruction, which consists in a dissolution of the protoplasts, always takes place with concentrations of 10^{-3} HA.

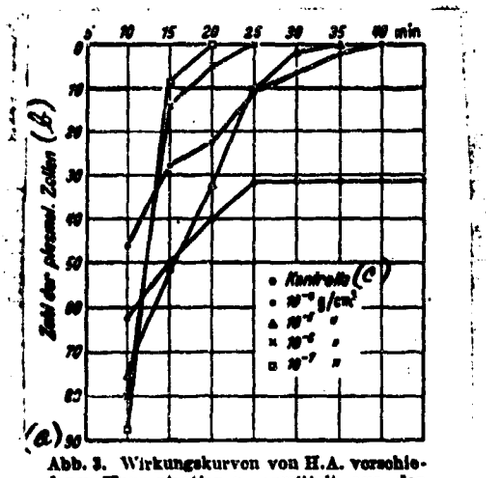

Abb. 3. Wirkungskurven von H.A. verschiedener Konzentrationen, ermittelt aus den Plasmolyseexeiten nach der Methode von KACZMAREK (Zahlen = Prozente).

(a) Fig. 3 - Curves showing the effects of HA in different concentrations; data represents deplasmolysis times using the Kaczmarek method (Figures in left-hand column represent percentages).

The following hypothesis was established as a general explanation of the overall behavior. Free HA increases viscosity, inhibits the intake of water and in concentrations of 10^{-3} destroys the protoplasm. On the other hand, bonded HA increases permeability to water either because it liberates auxin by displacing it, or because it turns itself into auxin. Both processes overlap one another. In concentrations of 10^{-4} the effect of the auxin is entirely overlapped by the powerful inhibiting action of the free HA. The 10^{-5} concentration shows a slightly promoting effect, which depends on the fact that the inhibition by HA is sharply reduced. In concentrations of 10^{-6} and particularly 10^{-7} the permeability-heightening effect

predominates and the inhibition disappers.

Oat coleoptile extract promotes water intake in concentrations which are comparable to the HA 10^{-5} to 10^{-7} concentrations.

Phenylacetic acid increases water permeability less than the above two mentioned growth factors; its optimum effectiveness is in the neighborhood of 10^{-5}. As in the case of HA, there is a reversal in the substance's action at 10^{-3}, where it has an inhibitory effect, while at 10^{-4} it has no effect at all. Thus the explanation given for HA may also be valid here, too. Colchicin behaves similarly. It promotes the maximum water intake in a concentration of 10^{-6}, while in 10^{-3} a powerful inhibition sets in. Ascorbic acid also acts in the same way. Its maximum promoting effect lies near 10^{-5}; in $10^{-3}$the protoplasm is destroyed. The growth promoting effect disappears both at 10^{-4} and at 10^{-8}. We were unable to discover any inhibitory action in the pure acid, but we did find such action present in sea buckthorn berry extract 10^{-2}, which behaves similarly otherwise.

It is common to all growth factors that they increase water permeability in specific dilutions (Fig. 4). With the exception of auxins taken directly from plant tissues (oat coleoptiles), the growth promoting effect is reversed at a certain, specific higher concentration - water permeability suddenly drops off sharply. This corroborates the fact that the explanation given above for HA is valid for all of these substances, namely, that they inhibit themselves, but to the extent that they form bonds with the protoplasm, they promote water intake, perhaps by displacing auxin. It must at least hold true that here two counteracting processes overlap one another. No inhibiting effect is ever noted to occur when using Avena extract, but that could be attributable to the fact that it is unfortunately impossible to use higher concentrations.

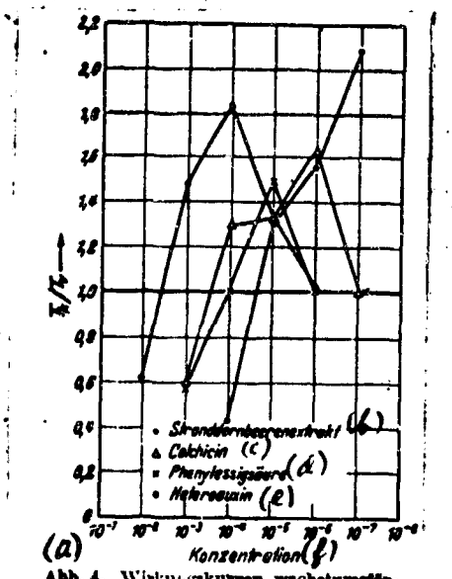

Abb. 4. Wirkungskurven wachstumsfördernder Stoffe von verschiedener Konzentration; ermittelt nach der Methode von DE HAAN.

(a) Fig. 4 - Growth curves of growth factors in varying concentrations; data taken using the De Haan method; (b) Sea buckthorn berry extract; (c) Colchicine; (d) Phenylacetic acid; (e) Heteroauxin; (f) Concentration.

Among typical inhibitors, above all coumarin, parasorbic acid and tomato extract, along with 4-hydroxycoumarin and ferulic acid were tested. Coumarin carries out a clear inhibitory action against water uptake in the range of concentrations between 10^{-3} and 10^{-5} (Fig. 5). The maximum effect occurs in the vicinity of 10^{-3}; this concentration sometimes leads to permanent plasmolysis. At no concentration does any reversal of action to a growth-promoting effect take place. Parasorbic acid behaves similarly. The greatest inhibition was observed at 10^{-3}, while the effect disappeared at 10^{-5}. In concentrations of 10^{-2} the protoplasts are destroyed. The destruction though, takes place entirely differently than with HA. While with HA the protoplasts dissolve and run out, with parasorbic acid, they burst, instead. In strong concentrations tomato extract powerfully inhibits water intake, as do 10^{-4} 4-hydroxycoumarin and 10^{-3} ferulic acid.

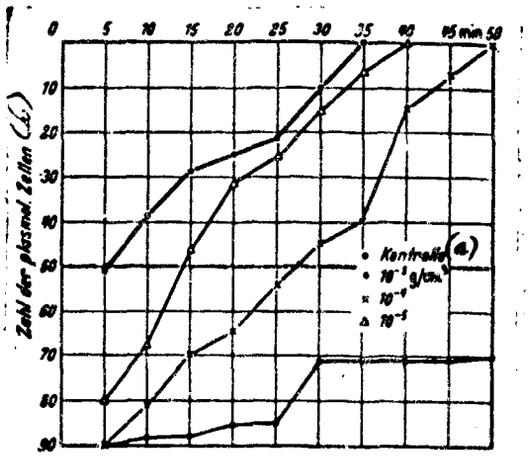

Abb. 5. Wirkungskurven von Cumarin verschiedener Konzentration; ermittelt aus den Plasmolysezeiten nach der Methode von KACZMAREK.

(a) Fig. 5 - Curves showing effects of coumarin in different concentrations; data represents deplasmolysis times using the Kaczmarek method; (b) Percentage of cells remaining plasmolyzed; (c) Controls.

Common to all these inhibitors is the fact that they decrease water permeability (Fig. 6). Unlike the above mentioned growth factors, they never undergo any reversal of their action, i.e., they do not promote water intake in any concentration. They rather continue to increase in inhibitory power with increase in concentration until they reach a certain maximum. Ethylene is a special case since it very powerfully inhibits elongation, but increases water permeability. Its greatest promotion of water permeability lies in the range of concentrations from 10^{-5} to 10^{-8}, with a maximum at 10^{-7}. There is no concentration of ethylene at which any decrease in water permeability occurs. We found in earlier investigations that ethylene partially deactivates auxin. It is no wonder, then, that no growth occurs in spite of the observed increase in permeability. The inhibition of growth together with thickening of the organs presumably depend on the fact that inhibitors are diffused throughout as a result of the increase in permeability, there being no antagonist available to counteract them.

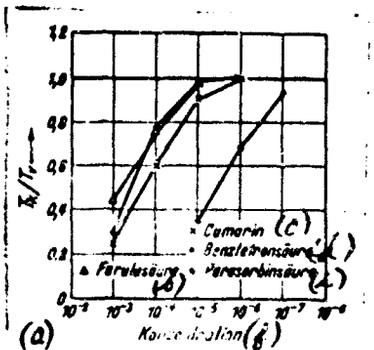

Abb. 6. Wirkungskurven wachstumshemmender Stoffe von verschiedener Konzentration; ermittelt nach der Methode von DE HAAN.

(a) Fig. 6 - Curves showing effects of growth inhibitors in different concentrations; data taken using the De Haan method; (b) Ferulic acid; (c) Coumarin; (d) 4-hydroxycoumarin; (e) Parasorbic acid; (f) Concentration.

Penicillin and 2, 4-dichlorphenoxyacetic acid have no effect on water permeability.

Potassium chloride was found to increase permeability between 10^{-3} and 10^{-6}, the increase becoming greater as concentration diminished; 10^{-2} and 10^{-7} took no effect. With the use of $CaCl_2$, complicated relationships were present, since in concentrations of 10^{-2} and 10^{-3} the permeability decreases with only a slight increase in concentration, while 10^{-1} promotes water intake; the effect disappears at 10^{-4}.

Tests carried out using various agents in combination gave the following results: On mixing KCl and $CaCl_2$ in the most promoting and most inhibiting concentrations, respectively, their effects were practically cancelled out. Since the Ca had a stronger inhibiting effect when tested separately than the promoting effect of K, the permeability promoting effect gave evidence of predominating. Also when one combines promoting KCl with inhibiting 10^{-4} HA concentration, no simple summation takes place, but, instead, the promoting action takes a more powerful effect than one would expect on the basis of calculations. The same is true for combinations of inhibiting coumarin and promoting HA or auxin. The simultaneously applied substances work as antagonists, it is true, but the promoting substance shows clear dominance. Consequently one comes to

the opinion that both substances exert their action at the same site and interfere with one another's mode of action at that point. Simple summation of the effects would speak in behalf of different sites of action for the different substances, as in such a case the separate processes could run their course without interfering with one another. This appears to be true only of ethylene when one combines this powerful permeability promoting substance with the inhibiting 10^{-4} HA concentration. In this case there is a pure summation and a cancelling out of both effects. This will be understandable if one assumes that ethylene on being uninhibited, continues to promote and that HA also when not counteracted continues to inhibit. If, instead, one combines ethylene with the promoting 10^{-7} HA, no further pure summation takes place, but instead there is only a slightly increased total promotion. This would favor the assumption that 10^{-7} HA's action consists in an activation of auxin. When the experiments are carried out using substances in combination this cannot be brought out because ethylene at least partially blocks the freed auxin. It must therefore be emphasized that with our limited knowledge of protoplasm structure, one must exercise the greatest caution in attempting to explain the results of such combined experiments. The opinions expressed herein in this regard, therefore, must be taken as having value only as speculation.

On the whole it was proven that when applied in physiologically effective concentrations growth factors increase the water permeability of protoplasm and growth inhibitors decrease its water permeability. The rapid and liberal water intake of cells during elongation is made possible directly by the growth factors while growth inhibitors act as antagonists and have a powerful braking action on water intake. The rapid initiation of this effect is clear evidence that it is a primary effect of said growth factors and inhibitors, which thus act first of all as regulators of water permeability. It is clear that even knowing this, the specific effect which auxin has on the cell membrane during the changes which follow still remains unexplained. We nevertheless look upon this as an advancement of knowledge to have shown that these various substances operate fundamentally on the protoplasm and not on the cell wall. The proof, too, that 10^{-3} to 10^{-4} HA destroys the protoplasts seems to us to be important. Accordingly, it should be assumed that all those effects which are to be observed in applying such high concentrations represent indirect effects of the HA. The liberation of "necrohormones"

could be involved here, as Haberlandt has assumed to be the case.

BIBLIOGRAPHY

Akkerman A.M. and W. Veldstra, Rec. Trav. chim. Pays-Bas, 66 (1947)

Avery, Jr. G.S., I. Berger and R.O. White, Amer J. Bot., 32 (1945)

Bärlund H., Acta bot. fenn., 5, 1 (1929).

Bein N., R. Signer and W.H. Schopfer, Experentia, 3 (1947), 291.

Borgström G., Kungl. Fysiograf. Sällskap. i. Lund, Forhandl., 9 (1939)

Borris H., Jb. Bot., 85 (1937 and 91 (1943).

Borris H., Die Ernahrung der Pflanze (Plant Nutrition), 1939, p. 35.

Brauner L., and M. Hasman, Bull. Fac. Med. Istanbul, 12 (1949)

Commoner B., S. Fegel and W.H. Muller, Amer. J. Bot., 30 (1943)

Fitting H., Jb. Bot., 56, 1 (1915) and 59, 1 (1919)

Friedrich G., Planta 25, (1936), 607.

Geiger-Huber M., and E. Sutter, Verh. der Schweiz. Naturforsch. Ges., 1943

Gellhorn E., Protoplasma (Berl. 12, (1930), 66.

Guttenberg H.v., Fortschr. Bot., 5 (1936)

Guttenberg H.v., Naturwiss, 30 (1942), 109.

Guttenberg H.v., and L. Krŏpelin, Planta, 35, (1947), 257.

Guttenberg H.v. and E. Lehle-Joerges, Planta, 35: (1947), 17.

Guttenberg H.v. and E. Steinmetz, Pharmazie, 2, (1947), 17.

Guttenberg H.v. and E. Steinmetz, Fortschr. Bot., 12 (1949), 323.

De Haan, I, Protoplasma (Berl.), 24, (1935), 186.

Heyn A., Rec. trav. bot. nëerl., 28 (1931), 113.

Huber B. and H. Höfler, Jb. Bot., 73 (1930), 351.

Kaczmarek A., Protoplasma (Berl.), 6 (1929), 209.

Kögl F., A. Haagen-Smit and H. Erxleben, Hoppe-Seylers Z., 228 (1934).

Koningsberger V., Nederl. Acad. de Wetensch., 9, No. 13 (1947).

Krŏpelin L., Dissertation Univ. Rostock (typewritten manuscript), 1945.

Kuhn R., and D. Jerchel, Naturwiss., 31 (1943), 468.

Larsen P., Planta, 30 (1939), 163.

Mairold F., Protoplasma (Berl.), 37 (1943), 445.

Moewus F., Biol. Zbl., 68 (1949), 58.
Overbeck F., Z.Bot., 18 (1926), 401.
Overbeek, I. van, Amer. J. Bot., 31 (1944).
Pohl R., Planta, 38 (1950), 324.
Raadts E., Planta, 36 (1949), 103
Reese G., Planta, 38 (1950), 321.
Reinders D., Rec. trav. bot. néerl., 39 (1942)
Reinert J., Z. Naturforschg., 56 (1950).
Ruge U., Z. Bot., 31, 1 (1937).
Ruge U., Planta, 35 (1947), 297.
Söding H., Jb. Bot., 74 (1931), 127.
Strugger S., Praktikum der Zell-und Gewebephysiologie der Pflanze (Practice of Plant Cell and Tissue Physiology), Berlin, 1935.
Sutter E., Ber. schweiz. Bot. Ges., 54 (1944), 197.
Umrath K. and F. Weber, Protoplasma (Berl.), 37 (1943), 522.
Veldstra H., Enzymologia (The Hague), 11 (1944), 137.
Veldstra H. and E. Havinga, Rec. trav. chim. Pays-Bas, 62 (1943), 842.
Weissenböck K., Phyton, 2 (1950).
Went F., Rec. trav. bot. néerl., 25, 1 (1928).
Wildman S. and J. Bonner, Amer. J. Bot., 35 (1948), 740.
Wurgler N., Experientia, 3 (1947), 416.

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